AMERICAN SOCIETY FOR MICROBIOLOGY | Applied and Environmental Microbiology

A Clonal Lineage of *Fusarium oxysporum* Circulates in the Tap Water of Different French Hospitals

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ABSTRACT

***Fusarium oxysporum* is typically a soilborne fungus but can also be found in aquatic environments. In hospitals, water distribution systems may be reservoirs for the fungi responsible for nosocomial infections. *F. oxysporum* was previously detected in the water distribution systems of five French hospitals. Sixty-eight isolates from water representative of all hospital units that were previously sampled and characterized by translation elongation factor 1α sequence typing were subjected to microsatellite analysis and full-length ribosomal intergenic spacer (IGS) sequence typing. All but three isolates shared common microsatellite loci and a common two-locus sequence type (ST). This ST has an international geographical distribution in both the water networks of hospitals and among clinical isolates. The ST dominant in water was not detected among 300 isolates of *F. oxysporum* that originated from surrounding soils. Further characterization of 15 isolates by vegetative compatibility testing allowed us to conclude that a clonal lineage of *F. oxysporum* circulates in the tap water of the different hospitals.**

IMPORTANCE

We demonstrated that a clonal lineage of *Fusarium oxysporum* inhabits the water distribution systems of several French hospitals. This clonal lineage, which appears to be particularly adapted to water networks, represents a potential risk for human infection and raises questions about its worldwide distribution.

Nosocomial infections caused by members of the genus *Fusarium* have emerged over the past 2 decades (1, 2). Infections caused by *Fusarium* isolates may be superficial, such as keratitis and onychomycosis, but *Fusarium* spp. can also cause life-threatening disseminated infections in immunocompromised patients (3). The *Fusarium* species most often involved are the morphological species *Fusarium solani* and *Fusarium oxysporum* (4, 5). These morphological species are now recognized as species complexes due to their high level of phylogenetic diversity (6, 7).

Members of the genus *Fusarium* have a diverse ecology that is linked to an impressive metabolic diversity together with a high genetic diversity. They are primarily known as saprophytes in the environment and are usually found in all types of soils (8–10), and they are frequently associated with plants as saprophytes, endophytes, or pathogens (9, 11). Fusaria may also be found in aquatic habitats, including natural water environments (12, 13) and drinking water distribution systems (14–17).

Water distribution systems in hospitals were identified as potential reservoirs for species of *Fusarium*, where they are thought to be responsible for nosocomial infections (14, 18). Fusaria in water may be aerosolized into the air when the water passes through taps and showers and then inhaled by immunocompromised patients (14). In two previous studies, we detected *F. oxysporum* in the water distribution systems of different French hospitals, with high concentrations of up to 10^5 propagules liter^{-1} in some units (19, 20). Heterogeneous distributions according to building and variability over time were explained by episodic detachments of parts of colonies from biofilms in the pipes. Strains of *F. oxysporum* were shown to be particularly adapted to the specific conditions offered by the complex water systems of hospitals (20). Analysis of partial sequences of translation elongation factor 1α (TEF1) showed low genetic diversity within these strains. Most of the isolates collected from water displayed the same sequence, even those from hospitals in two distant cities, suggesting the existence of a widespread clonal lineage in the water networks of hospitals (19, 20). The question then arose as to the origin of this lineage. Since it is able to grow in soil as well as soilborne isolates, it may have originated from surrounding soils.

The nuclear ribosomal DNA intergenic spacer (IGS rDNA) region has high sequence variation and may resolve much more genetic diversity than TEF1 at the intraspecific level (6). Microsatellites are also highly polymorphic loci with rapid mutation rates and are thus powerful means to characterize intraspecific diversity within fungi and to discriminate closely related isolates (21). Such microsatellite markers were developed to study the diversity of *F. oxysporum* populations (22). In addition, vegetative compatibility

Received 27 June 2016 **Accepted** 23 August 2016

Accepted manuscript posted online 23 September 2016

Citation Edel-Hermann V, Sautour M, Gautheron N, Laurent J, Aho S, Bonnin A, Sixt N, Hartemann P, Dalle F, Steinberg C. 2016. A clonal lineage of *Fusarium oxysporum* circulates in the tap water of different French hospitals. Appl Environ Microbiol 82:6483–6489. doi:10.1128/AEM.01939-16.

Editor: D. Cullen, USDA Forest Products Laboratory

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Supplemental material for this article may be found at http://dx.doi.org/10.1128/AEM.01939-16.

TABLE 1 Origin and molecular characterization of the isolates of *Fusarium oxysporum* from water analyzed in this study

Hospital	Unit	Location	No. of isolates	TEF1 sequence type[a]	Size of Fol624 locus (bp)	Size of CH2-15 locus (bp)	No. of isolates characterized using IGS	IGS sequence type
D1	Internal medicine	Room 382	15	1	160	190	1	1
		Room 380	2	1	160	190	1	1
		Room 355	3	1	160	190	1	1
			1	1	160	0	1	1
		Room 374	2	1	160	190	1	1
	Digestive surgery	Room 284	3	1	160	190	1	1
			1	2	155	190	1	2
		Room 282	1	1	160	190	1	1
		Room 286	3	1	160	190	1	1
	Adult hematology	Room 38	2	1	160	190	2	1
D2	Rheumatology	Room G032A	11	1	160	190	1	1
			2	1	160	0	2	1
D3	Geriatric unit	Room C230	13	1	ND[b]	ND	12	1
N1	Adult building	Water inlet	1	1	160	190	1	1
	Cardiology	Room E40	2	3	150	185	2	3
	Laundry	Shower 1-6	1	1	160	190	1	1
			2	1	ND	ND	1	1
	Mortuary	Fountain	1	1	ND	ND	1	1
	Vascular surgery	Common bathroom	1	1	ND	ND	1	1
N2	Medical intensive care	Room 13	1	1	ND	ND	1	1

[a] Translation elongation factor 1α (TEF1) sequences were obtained by Sautour et al. (19) and Steinberg et al. (20).
[b] ND, not determined.

testing has been extensively used to evaluate genetic diversity in phytopathogenic populations of *F. oxysporum* and to assign isolates to vegetative compatibility groups (VCGs) (23, 24). Vegetatively compatible isolates, which are able to anastomose, can be identified by the complementation of nitrate-deficient mutants (25). A VCG comprises closely related isolates that are descended from a common ancestor and represents a clonal unit within *F. oxysporum* (26).

The present study was initiated to determine (i) whether the isolates collected from the different water systems in French hospitals represent a clonal lineage and (ii) whether the isolates collected from water originate from soil. The first question was addressed by characterizing the genetic diversity of the isolates by IGS sequencing, microsatellite analysis, and VCG testing. The second question was addressed by comparing the genotypes of the isolates collected from water with those of *F. oxysporum* inhabiting surrounding soils.

MATERIALS AND METHODS

***Fusarium oxysporum* isolates.** This study included 68 isolates of *F. oxysporum* that were collected from the water distribution systems of different hospitals located in two cities in eastern France, Dijon and Nancy, which lie 230 km apart. Forty-six isolates were collected from February to September 2009 in hospitals D1 and D2 located in Dijon (19), and 22 isolates were collected from October 2009 to October 2010 in hospital D3 located in Dijon and hospitals N1 and N2 located in Nancy (20) (Table 1). The 68 isolates were selected from 232 isolates that were previously characterized to be representative of all units sampled in the different hospitals and at all sampling times (see Table S1 in the supplemental material).

Molecular characterization. With the exception of three previously analyzed isolates, all of the isolates displayed identical TEF1 sequences (19, 20). The isolates were further characterized using microsatellite markers that were developed by Cheng-Hua Huang for populations of *F. oxysporum* f. sp. *radicis-lycopersici* (22). Among the 27 microsatellite loci proposed (22), we selected the following five loci with high numbers of alleles: FOL20 (forward primer, CATTGAGGAAGAGCGGAAAG; reverse primer, CACATTTGGCACAGCAATCT), FOL35 (forward primer, GTCGTTTTCAAGGACGCACT; reverse primer, GGTGGCAGTTTCCTCCTTTT), FOL296 (forward primer, CACTGAAGGAAATGCAGCAG; reverse primer, TAGGCTCTGGAGATGCTTGG), FOL624 (forward primer, CAAGAGGCCAGCGATAGTGT; reverse primer, AGCTTTTGATACCCCATTCG), and CH2-15 (forward primer, ATCTTCCTCACGGTTTTGGA; reverse primer, TGTAGCGTAGCACAACAGTGG). For each microsatellite locus, PCR amplification was performed in a final volume of 20 μl, containing 10 ng of DNA, 0.15 μM each primer, 50 μM each deoxyribonucleotide triphosphate (dNTP), 2.5 mM $MgCl_2$, 1 U of *Taq* polymerase (MP Biomedicals, Illkirch, France), and 1× PCR buffer. Amplification consisted of an initial denaturation step of 3 min at 94°C, followed by 35 cycles of 30 s at 94°C, 30 s at 52°C, 45 s at 72°C, and a final extension of 20 min at 72°C. Strain MIAE00045 of *F. oxysporum* f. sp. *radicis-lycopersici* was used as the positive control in each PCR experiment. Aliquots (10 μl) of PCR products were checked by electrophoresis on a high resolution 4% NuSieve 3:1 agarose (FMC, Rockland, ME, USA) gel with a slow migration for 150 min. The sizes of the PCR products were determined by comparison with molecular weight marker VIII (Roche Diagnostics, Meylan, France).

Some isolates were further compared by sequencing nearly full-length nuclear ribosomal DNA intergenic spacers (IGSs). The IGS regions were amplified by PCR using primers NL11 (CTGAACGCCTCTAAGTCAG) and CNS1 (GAGACAAGCATATGACTAC) (6). PCR amplification was performed in a final volume of 50 μl, containing 10 ng of DNA with 0.2 μM of each primer, 100 μM each dNTP, 1.5 mM $MgCl_2$, 5 U of *Taq* polymerase (MP Biomedicals), and 1× PCR buffer. Amplification consisted of an initial denaturation step of 5 min at 94°C, followed by 35 cycles

of 1 min at 94°C, 1 min at 55°C, 1 min at 72°C, and a final extension of 5 min at 72°C. Two PCR amplifications were performed for each isolate. Three-microliter aliquots of PCR products were checked by electrophoresis. The PCR products were sequenced by Beckman Coulter Genomics (Takeley, United Kingdom) using primers NL11, NLa (TCTAGGGTA GGCKRGTTTGTC), CNSa (TCTCATRTACCCTCCGAGACC), and CNS2 (AACTTCAATCGCCTCTCACG) (6). The eight sequences that were obtained for each isolate (two PCR products per four primers) allowed us to cover the complete sequence twice; they were assembled using SeqMan 6.0 (DNAStar Lasergene; GATC Biotech, Constance, Germany) and aligned using CLUSTAL_X.

Vegetative compatibility testing. Vegetative compatibility was determined by pairing complementary nitrate-nonutilizing (*nit*) mutants derived from each strain analyzed as previously described (25). Briefly, mutants were first generated for each strain on potato dextrose agar-potassium chlorate medium (PDA, 30 g liter^{-1} and $KClO_3$, 15 g liter^{-1}). Plates were incubated for 5 to 15 days at 25°C. Chlorate-resistant colonies were transferred to minimal medium (MM) (28) and incubated for 3 to 7 days at 25°C. The *nit* mutants with very thin growth on MM were then classified as *nit1*, *nit3*, or *nitM* based on their phenotype on minimal medium (MM) containing one of the following three different nitrogen sources: nitrate ($NaNO_3$, 2 g liter^{-1}), nitrite ($NaNO_2$, 1 g or 0.5 g liter^{-1}), or hypoxanthine (0.2 g liter^{-1}). For some isolates for which the different types of mutants could not be obtained, the concentration of potassium chlorate in the medium was increased from 15 g liter^{-1} to 30 g liter^{-1}. If this was still not enough, the mutants were generated on KMM medium. KMM comprised MM amended with 1.6 g liter^{-1} asparagine and 15 g liter^{-1} $KClO_3$ (28).

Complementary *nit* mutants were paired on MM in 5-cm petri dishes, and vegetative compatibility was recognized by an aerial growth at the line of contact between the two paired mutants, corresponding to the formation of a heterokaryon. Control pairings between complementary mutants that were derived from the same parental strain were made to detect any strain that might be heterokaryon self-incompatible (HSI) (25). Depending on the number of mutants that could be obtained for each strain, 3 to 7 *nitM* mutants were paired with 6 to 10 *nit1* mutants, leading to 30 to 70 pairings to determine self-incompatibility. Then, vegetative compatibility was determined by pairing complementary *nit* mutants that were derived from all isolates in all pairwise combinations. For each pairwise combination, six pairings were performed: (i) three *nit1* mutants of isolate A were paired with three *nit3* or *nitM* mutants of isolate B, and (ii) three *nit3* or *nitM* mutants of isolate A were paired with three *nit1* mutants of isolate B. The following two types of complementation results were obtained: a robust heterokaryon with a line of dense aerial growth at the hyphal contact zone of the mutants or a weak heterokaryon with only a thin zone with little aerial mycelium. In both cases, the corresponding strains were considered compatible.

Comparison of isolates from water and soilborne isolates of *F. oxysporum*. The genotypes of the 68 isolates from water were compared with those of 300 soilborne isolates of *F. oxysporum* that were collected in surrounding soils. In a previous study, Edel-Hermann et al. (29) analyzed *Fusarium* diversity in soils sampled at three different locations (E, B, and M) in eastern France near Dijon. The TEF1 sequences of 100 *F. oxysporum* isolates from each of the three soils were compared with the TEF1 sequences of isolates from water that were previously described (19, 20). Sequences were aligned and compared with the Kimura two-parameter distance model and the neighbor-joining (NJ) method using SeaView (30). The tree was rooted on the most divergent sequence. The topology of the resulting tree was tested by NJ bootstrapping with 1,000 resamplings of the data.

Accession number(s). The three IGS sequence types found in this study were deposited in GenBank under accession numbers KX759109 (reference isolate 474, MIAE00798), KX759110 (isolate 676, MIAE00799), and KX759111 (isolate N13, MIAE00935).

RESULTS

Molecular diversity of *Fusarium oxysporum* isolates collected from water. For three of the five microsatellite loci tested (FOL20, FOL35, FOL296), PCR amplification of fungal DNA yielded either no PCR product or several inconsistent PCR products. In contrast, the loci FOL624 and CH2-15 were amplified consistently, except for three isolates for which no PCR product was obtained with the CH2-15 primers. For locus FOL624, two fragments (230 and 240 bp) were obtained for the positive control and one fragment of 160 bp was obtained for all of the isolates from water associated with the dominant TEF1 sequence (sequence type 1 [ST1]). Two different alleles were also obtained: 155 bp for the isolate associated with TEF1 ST2 and 150 bp for the two isolates associated with TEF1 ST3 (Table 1). For locus CH2-15, a 195-bp fragment was obtained for the positive control and a 190-bp fragment was obtained for all of the isolates from water associated with TEF1 ST1 (Table 1). A different allele (185 bp) was found only for the two isolates associated with TEF1 ST3. To conclude, the same level of discrimination was obtained when using microsatellite genotyping and TEF1 sequences.

F. oxysporum isolates were further characterized using IGS sequences. The nearly full-length IGS sequences of 34 isolates were compared. Thirty-one isolates that originated from the five different hospitals in the two different cities had identical IGS sequences (ST1) (Table 1). The three isolates with different TEF1 sequences also showed different IGS sequences; the isolate associated with TEF1 ST2 was associated with IGS ST2, and the two isolates associated with TEF1 ST3 were associated with IGS ST3.

Identification of a clonal lineage. Fifteen isolates with identical TEF1 and IGS sequences (ST1) were further characterized by VCG testing (Table 2). Five isolates were found to be HSI. The 10 remaining isolates were paired in all pairwise combinations. The different pairings yielded either a robust heterokaryon, a weak heterokaryon with little aerial mycelium, or no heterokaryon at all. The six pairings performed for a given pairwise combination of two isolates did not yield the same result depending on the mutants paired (Table 3). Finally, the six pairings revealed six heterokaryons in some pairwise combinations, such as the pairing of isolates 474 and 922, but only one weak heterokaryon in some others, such as the pairing of isolates 875 and 922. The results of vegetative compatibility are summarized in Fig. 1. Two groups of three and five isolates were found, with all isolates in each group compatible with one another. Some isolates, but not all of those in one group, were compatible with one or all isolates in the other. The three remaining isolates were compatible with one to three isolates of the group of five isolates. To conclude, we only found one VCG among the 10 isolates, given that all isolates were compatible with at least one isolate in the group (Table 2).

Comparison of soilborne isolates and isolates from water. Sequence alignments revealed 12, 13, and 9 different TEF1 sequence types among the isolates collected from the E, B, and M soils, respectively. Finally, 22 different STs were identified among soilborne isolates (Fig. 2). Four STs (11, 12, 15, and 17) were common in the three soils, four STs (4, 6, 8, and 10) were common in two soils, and the 14 remaining STs were only found in one of the three soils. Comparison of isolates collected from soils and isolates collected from water showed that only ST2 was shared by two soilborne isolates and one isolate collected from water. In contrast, neither ST1, which corresponded to the dominant ST

TABLE 2 Isolates of *Fusarium oxysporum* characterized for vegetative compatibility group

Accession no.[a]	Isolate	Hospital of origin	Unit (location)	Date of isolation (day/mo/yr)	VCG testing[b]
MIAE00798	474	D1	Internal medicine (room 382)	17/02/2009	X
MIAE01433	859	D1	Internal medicine (room 355)	11/06/2009	HSI
MIAE01438	922	D1	Internal medicine (room 374)	17/09/2009	X
MIAE01423	553	D1	Digestive surgery (room 284)	26/02/2009	X
MIAE01428	600	D1	Digestive surgery (room 282)	12/03/2009	HSI
MIAE01437	920	D1	Digestive surgery (room 286)	10/09/2009	HSI
MIAE01435	875	D1	Adult hematology (room 38)	25/06/2009	X
MIAE01436	908	D1	Adult hematology (room 38)	23/07/2009	HSI
MIAE01418	219	D2	Rheumatology (room G032A)	12/02/2009	HSI
MIAE01439	924	D2	Rheumatology (room G032A)	24/09/2009	X
MIAE01398	N1	N1	Laundry (shower 1-6)	22/10/2009	X
MIAE00934	N3	N1	Adult building (water inlet)	05/11/2009	X
MIAE01402	N10	N1	Mortuary (fountain)	26/01/2010	X
MIAE01403	N20	N1	Vascular surgery (common bathroom)	20/07/2010	X
MIAE01404	N22	N2	Medical intensive care (room 13)	24/08/2010	X

[a] MIAE, microorganisms of interest for agriculture and environment (INRA, Dijon, France).
[b] VCG, vegetative compatibility group; HSI, heterocaryon self-incompatible; X, group of compatible isolates in one to six pairwise combinations.

among isolates from water, nor ST3 was found among soilborne isolates.

DISCUSSION

A surprisingly low level of genetic diversity was found among *F. oxysporum* isolates collected from the tap water of different hospitals. A combination of two-locus TEF1 and IGS rDNA sequencing and the microsatellite analysis revealed only three distinct genotypes, of which one was dominant within the analyzed collection. The two poorly represented genotypes included one isolate collected in hospital D1 and two isolates collected in hospital N1. The dominant genotype was detected in the five hospitals, situated in two different French cities, that were investigated in this study. VCG typing among the isolates that share this dominant genotype revealed variable results. In some pairwise combinations, all pairings produced a robust heterokaryon. In other pairwise combinations, the different pairings produced no heterokaryon, a weak heterokaryon, or a robust heterokaryon. Such differences in the intensity of the reactions have previously been reported for *F. oxysporum* (26, 31). Rather than strictly qualitative, the differences in heterokaryon reactions may be quantitative depending on the number of successful hyphal fusions (26). Both weak and robust heterokaryons are evidence of compatibility. In addition, the absence of heterokaryons may represent a false-negative reaction. It is clear from our results that a single pairing for one combination is not enough to conclude that two isolates are incompatible. In our study, VCG testing revealed two groups of three and five isolates. However, they did not correspond to discrete groups completely differentiated from the other isolates. Some reactions were positive between isolates of the two groups or between isolates of one group and one of the remaining isolates. Apart from the five isolates that were found to be HSI, none of the 10 isolates tested in all of the pairwise combinations was found to be completely differentiated from the others. On the contrary, all 10 isolates showed at least one positive reaction with one of the other isolates. Taking into account the quantitative interpretation of vegetative compatibility discussed above, we concluded that the 10 isolates, in fact, belonged to a unique VCG. Since vegetative compatibility is a good indication of clonal ancestry, this study showed that the water distribution system of the five different hospitals was colonized by the same clonal lineage of *F. oxysporum*. In addition, five isolates were identified as vegetatively self-incompatible. HSI isolates have already been reported in *F. oxysporum* (24, 32, 33); they represent one drawback of VCG typing since these isolates cannot be classified into VCGs. The five HSI isolates in our study may belong to the same clonal lineage deter-

TABLE 3 Results of vegetative compatibility testing among 10 isolates of *Fusarium oxysporum*[a]

	No. of heterokaryons and weak heterokaryons obtained per isolate combination									
Isolate	474	553	875	922	924	N1	N3	N10	N20	N22
474		0	0	6H	0	0	5H + 1WH	0	1H	5H
553			0	3WH	0	0	0	0	4H	6H
875				1WH	3H	0	0	0	0	0
922					0	0	5H + 1WH	0	2H + 3WH	6H
924						0	0	0	0	3H
N1							4H + 1WH	6H	1WH	0
N3								3H + 1WH	4H + 1WH	3H + 3WH
N10									1H	1WH
N20										5H
N22										

[a] The numbers of heterokaryons (H) and weak heterokaryons (WH) obtained among six pairings performed for each pairwise combination are presented.

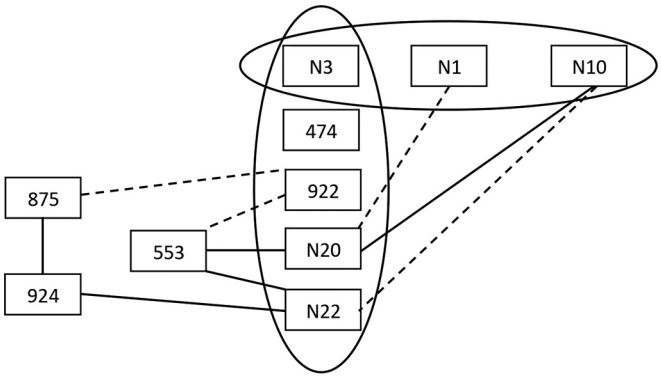

FIG 1 Schematic representation of vegetative compatibilities obtained among 10 isolates of *Fusarium oxysporum*. Pairing yielded one to six heterokaryons in all pairwise combinations between isolates grouped in the same circle and between isolates joined by a line. Pairing yielded one to three weak heterokaryons between isolates joined by a dotted line.

mined through VCG testing since they all displayed the same alleles for the analyzed microsatellite markers. Such markers are indeed powerful for fine-scale characterization of organisms at the intraspecific level (21).

In this study, we also investigated soilborne isolates of *F. oxysporum* to compare them with the clonal lineage found in the different water systems. With 22 different TEF1 sequence types, soilborne isolates of *F. oxysporum* were found to be more diverse than those collected from water. None of the 22 different TEF1 sequences was the same as the dominant TEF1 sequence associated with isolates collected from water. We also compared our data set with those obtained in two recent surveys of *F. oxysporum* diversity in soils based on TEF1 sequences. Balmas et al. (34) found 46 ST among 96 isolates that were collected from 24 different soils, and Laurence et al. (35) found 37 ST among 202 isolates that were collected from 10 different soils. Again, none of them corresponded to the ST of the isolates collected from water. This result is surprising since *F. oxysporum* is typically a soilborne fungus, and soil is generally considered the reservoir of diversity of this species. It is clear from our results that the clonal lineage of *F. oxysporum* dominating in the water environment is not encountered in soil. It may be present but minor in soils, or it may be specific to the water environment. Although it is able to grow in disinfected soil as well as soilborne isolates (20), it may not be competitive enough within the whole microflora. Unlike soilborne isolates, this clone of *F. oxysporum* has particular phenotypic characteristics that enable its survival and growth in a particular oligotrophic environment (20). Although *F. oxysporum* was sporadically collected from aquatic environments, such as the sea or rivers (12), surveys of tap

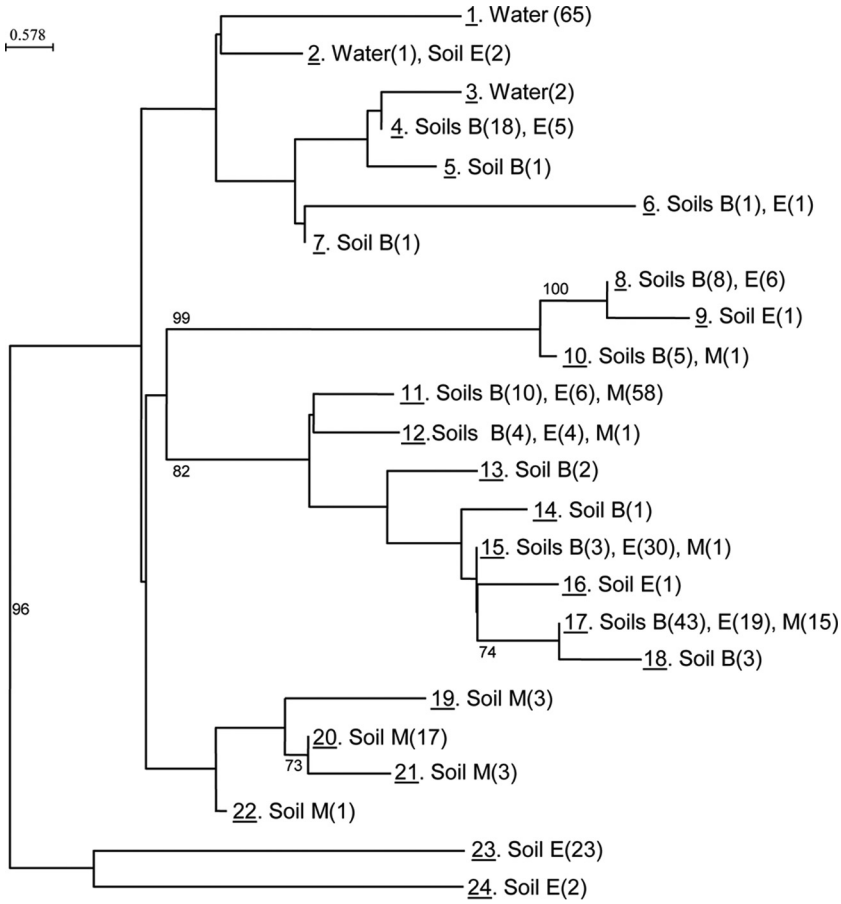

FIG 2 Neighbor-joining tree (Kimura two-parameter distance) of 24 translation elongation factor 1α (TEF1) sequence types (STs) of *Fusarium oxysporum* isolates collected from water and soil. Bootstrap values (>70%) are near the equivalent branches. For each ST, the origin of the isolates (water or soils B, E, or M) is indicated with the number of isolates in brackets.

water generally failed to isolate *F. oxysporum* from nonmedical buildings (20, 36, 37). In contrast, *F. oxysporum* was detected in the water systems of three geographically distant hospitals in Houston, TX, Seattle, WA, and Baltimore, MD, and all isolates had the same TEF1 and IGS sequences (ST33) (18) as those of the clonal lineage we described. Thus, this clone of *F. oxysporum* seems to be adapted to an aquatic environment whatever the geographic location. Since the same ST dominant in water was also associated with most clinical isolates, O'Donnell et al. (18) concluded that hospitals may serve as reservoirs for nosocomial fusarial infections. The same ST was also associated with most clinical isolates collected in Italy (38). Fortunately, no cases of human fusariosis due to *F. oxysporum* were reported in the French hospitals of Dijon and Nancy, where *F. oxysporum* isolates that were analyzed in this study were collected in 2009 and 2010. However, it is quite worrying to discover that clinical and aquatic isolates are similar in different hospitals investigated worldwide. Besides the pathogenicity and the risk of infection of immunocompromised patients, one of the most intriguing aspects of this study, given the published results noted above, is the fact that it is a clonal lineage that may be distributed worldwide but that is detected only in hospital settings, particularly in water distribution networks of these hospitals. If this clonal lineage had been detected in drainage water, it would have been possible to conclude that contamination of the water was due to patients while, in fact, the opposite may be true. Indeed, fungal isolates detected in the water system are a potential risk for immunocompromised patients. Since they may be aerosolized into air and inhaled by patients, it is essential to implement prophylactic measures in hospitals to control invasive fusariosis by using filters on taps and showers. The next step of our work will be to perform pathogenicity tests on human cells or tissues to determine whether the aquatic isolates of *F. oxysporum* can be responsible for infections. More generally, in terms of epidemiology and evolution, it would be interesting to understand the mechanisms of selection, whether anthropogenic or natural, that led to the worldwide distribution of this clonal lineage.

FUNDING INFORMATION

This work, including the efforts of Alain Bonnin, was funded by Programme Hospitalier de Recherche Clinique 2008 (PHRC interrégional, Délégation de la Recherche Clinique de Bourgogne, Dijon, France).

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